

# Natural polypeptides in left-handed helical conformation

## A circular dichroism study of the linker histones' C-terminal fragments and $\beta$ -endorphin

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Circular dichroism has been used to investigate the histone H1 and H5 C-terminal fragments and  $\beta$ -endorphin conformation. It has been shown that in aqueous solution these polypeptides preferably adopt the left-handed helical conformation of the poly-L-proline II type. A break in the linear temperature dependence of the CD value was found in the temperature interval between 50 and 55°C. It was proposed to be due to non-cooperative disordering of the conformation caused by the destruction of the hydration shell.

Linker histone;  $\beta$ -Endorphin; Circular dichroism; Poly-L-proline II conformation; Non-cooperative melting

### 1. INTRODUCTION

We have previously studied the conformational peculiarities of histones and polypeptide hormones by circular dichroism (CD) and have shown the presence of an extended left-handed helix of the poly-L-proline II type (PPII) [1–3]. Evidence in favour of the presence of a left-handed PPII helix has been obtained by studying CD spectra of the charged poly-L-lysine at low (down to –100°C) temperatures [4] and by vibrational CD spectroscopy [5]. The PPII type structure has been revealed in globular proteins [6,7], in the terminally blocked amino acids [8] and in predominant amounts in glycoproteins [9]. The analysis given in the review by Woody [10] suggests that the PPII conformation plays a significant role in peptide and protein systems.

We have carried out an investigation of C-terminal fragments of H1 and H5 linker histones, free from aromatic amino acids, and of the peptide hormone  $\beta$ -endorphin. Eukaryote chromatin linker histones consist of three domains: a globular central, a short N-terminal and a lengthy C-terminal domain, which binds tightly to the internucleosomal DNA [11]. The latter is enriched in Lys, Arg and Pro residues and devoid of any standard secondary structure [12–14].  $\beta$ -Endorphin is the 31-residues long C-terminal fragment of  $\beta$ -lipotropic hormone with opiate-like activity [2].

### 2. MATERIALS AND METHODS

The total fraction of lysine-rich histones was obtained from calf thymus [15]. Histone H1 was isolated by ion-exchange chromatography on CM-cellulose Whatman CM-32 and SP-Sephadex C-50. The H1 C-terminal fragment (residues 123–213) was obtained by thrombin digestion at Lys<sup>122</sup> followed by ion-exchange chromatography on SP-Sephadex C-50. The H1/H5 histone fraction was isolated from chicken erythrocytes [16] and histone H5 was purified by ion-exchange chromatography on CM-cellulose. It was hydrolysed with acetic acid at Asp<sup>99</sup> [13], and its C-terminal fragment (residues 100–189) was purified by gel filtration on Sephadex G-75. Histone fragments were studied in a 0.01 N HCl aqueous solution, pH 2.0, as in [12–14]. The concentration of fragments (devoid of aromatic residues), determined by UV absorption at 230 nm ( $E_{1\%}^{1\text{cm}} = 2.38$ ) and by a colorimetric method [17], was equal to 0.6–0.8 mg/ml. The polypeptide composition of the samples was analyzed by SDS-PAGE on 12.5% gels.

The porcine  $\beta$ -endorphin, isolated from the pituitary glands [18], was a gift of Prof. Yu.A. Pankov (Institute of Hormone Chemistry, Moscow). It was dissolved in bidistilled water and its concentration, determined by weight (after drying over P<sub>2</sub>O<sub>5</sub>) and also spectrophotometrically [2] was equal to 0.2–0.4 mg/ml.

The CD spectra were measured on a Mark III Jobin-Yvon dichrograph (France). Heating of the solutions was performed at a constant rate of 30°C/h. Electrophoresis was carried out before and after every experiment to check for the absence of proteolytic peptide degradation. Peptide films were prepared as in [2]. CD was expressed in  $\Delta\epsilon$  units ( $1\text{cm}^{-1}\cdot\text{mol peptide}^{-1}$ ).

### 3. RESULTS AND DISCUSSION

Fig. 1 and 2 show the CD spectra of aqueous solutions of histone fragments and  $\beta$ -endorphin at different temperatures. In all cases a large minimum near 200 nm, a maximum near 220 nm (at low temperatures) and a slight minimum close to 230 nm are observed. For the histone fragments the maximum at 220 nm at low tem-

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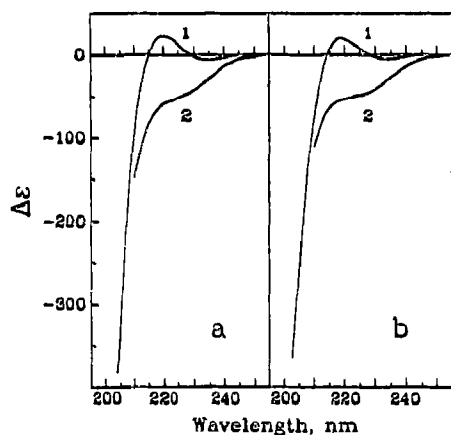


Fig. 1. CD spectra of histone fragments in 0.01 N HCl aqueous solution, pH 2.0, at various temperatures. Histone H1 fragment 123-213 (a): 1, 5°C; 2, 60°C; histone H5 fragment 100-189 (b): 1, 6°C; 2, 77°C.

perature is a positive one while for  $\beta$ -endorphin at the same temperature it is still in the negative ellipticity region. Heating results in the smoothing and disappearance of the 220 nm maximum, while cooling of the samples leads to the complete restoration of their spectra.

These CD spectra are typical of the PPII conformation [3,10], which means that considerable regions of the left-handed helix are present in the polypeptides at low temperatures. The structure is labile, becomes loosened on heating and is gradually destroyed [2], displaying an isodichroic point at 201.5 nm, as seen in CD spectra of the  $\beta$ -endorphin solution in the temperature interval 2-57°C (Fig. 2). Upon aggregation it may be transformed, for instance, into  $\alpha$ -helix, producing characteristic  $\alpha$ -helix minima, as in  $\beta$ -endorphin film (Fig. 3).

We have shown earlier that for aqueous solutions of  $\beta$ -endorphin and histone H5 C-terminal fragment there was a break in the linear temperature dependence of

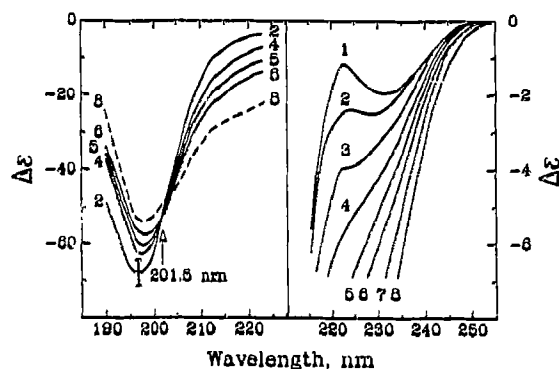


Fig. 2. CD spectra of  $\beta$ -endorphin in water at various temperatures: 1, 2°C; 2, 10°C; 3, 20°C; 4, 30°C; 5, 40°C; 6, 48°C; 7, 67°C; 8, 85°C. Left panel: 190-225 nm region; 201.5 nm, the isodichroic point in the temperature interval 2-57°C; vertical bar, experimental error. Right panel: 215-255 nm region.

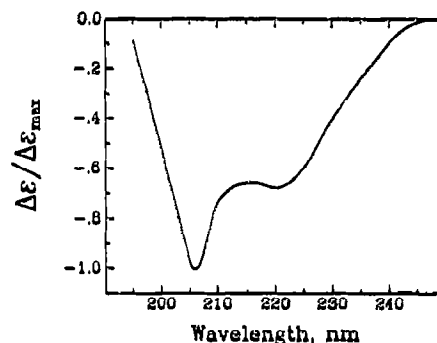


Fig. 3. Normalized CD spectrum of  $\beta$ -endorphin film, precipitated from aqueous solution at ambient humidity and temperature.

partial heat capacity within a narrow temperature interval which then reached a plateau [19]. Continuous measurement of the course of CD value temperature dependence at the extremum near 220 nm shows that for all three peptides the linearity, characteristic for the left-handed helix [2], is lost near 50-55°C (Fig. 4). The linear extrapolation of CD curves before and after this region (Fig. 4, dashed lines) gives the following values for the inflexion points: H5 fragment ( $49 \pm 1$ )°C, H1 fragment ( $54 \pm 1$ )°C,  $\beta$ -endorphin ( $56 \pm 1$ )°C. For the latter the inflexion region coincides with the disappearance of the isodichroic point of the CD spectra (Fig. 2). The inflexion points for the H5 fragment and  $\beta$ -endorphin, within the limits of measurement error, are close to those obtained for the microcalorimetric curves [19]. A similar high temperature break in the linear CD temperature dependence could be seen at the CD melting curve (4-80°C) of a polylysine water solution (pH 7.6, PPII conformation) [4].

The presence of the isodichroic point for  $\beta$ -endorphin in a definite temperature interval shows that a given family of CD spectra is a linear combination of two spectra, i.e. the temperature change results in a transition between two states, one of which predominates at

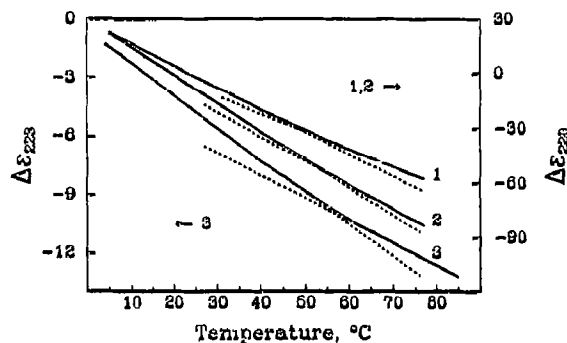


Fig. 4. Continuous measurement of the CD value temperature dependence for aqueous solutions of the histone H5 fragment 100-189 (1), histone H1 fragment 123-213 (2) and  $\beta$ -endorphin (3). The dashed lines are the linear extrapolation of the CD value before and after the inflexion points. Measurement errors do not exceed thickness of the curves.

low temperatures, and the other prevails at high temperatures. These basic spectra evidently correspond to the spectrum of the PPII conformation and to that of the polylysine 'high-temperature' form, which has been observed for polylysine solutions above 63°C or with 5 M NaCl [4]. The predominating of this conformational form after the inflexion region could explain the retaining of the relatively minor CD temperature dependence, similar to that observed for polylysine [4].

Temperature increase results in a disturbed isodichroicity and broken linearity of the ellipticity (Fig. 4) and partial heat capacity [19] temperature dependences. This is an indication of the pronounced changes in the solution properties of the polypeptides, in spite of the absence of a heat absorption peak during microcalorimetry. The interaction with the structural water molecules contributes much to the PPII helix stabilization [2,10]. The non-cooperative destruction of its hydration shell in the absence of intra-chain hydrogen bonds finally results in the elimination of all PPII containing regions.

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